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STUDIES ON EPIDERMAL REGENERATION BY MEANS OF THE STRIP METHOD*

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The medical use of cellophane tape goes back many years. Pediatricians have used cellophane tape to examine the anal mucosa for pin worm ova with considerable success. Wolf (17, 18) in 1939 first used cellophane tape for the systematic removal of keratin cells from the skin surface and described in detail the microscopic appearance of the cells that adhered to the tape. Szakall (15, 16) used the method for morphologic and chemical examination of the stripped off keratin. However, it was not until 1951 that Pinkus (9) made the discovery that multiple applications of cellophane tape caused a predictable mitotic response in the epidermal cells. This method consists of applying successively, multiple strips of cellophane tape to the same area and removing the keratin cells, layer by layer. Sections taken at varying intervals show a burst of mitoses in basal and lower prickle cell layers of the epidermis, 48–72 hours after stripping. One half to 12 hours after keratin stripping the stratum corneum which is normally present in histological sections has been removed. The stratum granulosum also is usually lost (10). The stratum malpighii contains many pyknotic cells. The basal cells are swollen and hypertrophic and may make up one third or more of the thickness of the epidermis. At 24 hours, a parakeratotic layer begins to form on the surface. At 48 hours, the stratum granulosum is visible beneath the layers of parakeratotic cells and in the basal and lower prickle cell layer many mitoses are found. At 72 hours a new anuclear horny layer is evident and the parakeratotic layer is pushed up to be sloughed off. The peak of the mitotic response is usually reached in 48 hours. However, this seems to be dependent on the degree of cellular damage. If the stripping process causes too much cellular damage there is a delay in the peak of mitosis which may not occur until 72 hours. During this time the corium reflects the cellular unrest above it by a perivascular infiltrate composed mainly of lymphocytes around the capillaries in the dermis.

The present investigation is an attempt to shed some light on the mechanisms playing a role in the forced epidermal regeneration. A triple approach was used.

A. The relative importance of loss of keratin cells and of cellular trauma due to dehydration following the stripping procedure was investigated. It was suggested that covering the area may presumably decrease dehydration and thus minimize the effects due to loss of the stratum corneum.

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B. Mitotic activity in normal skin adjacent to the stripped area was studied. Pinkus (9, 10) suggested that an overflow of mitotic activity may occur around an injured area.

C. Histochemical technics were employed. Lobitz (3) showed that there is a marked accumulation of glycogen in the epidermal cells after the keratin stripping stimulus.

Acid phosphatase is found in abundance in the epidermal cells. Although the significance of its presence is not known, it has been suggested that it may be involved in the keratinization process.

Succinic dehydrogenase is one of the enzymes associated in the tricarboxylic acid cycles of Krebs. This enzyme is intimately involved in cellular respiratory processes.

Studies were undertaken to demonstrate any change in the concentration of acid phosphatase and succinic dehydrogenase in the rapidly proliferating epidermal cells after stripping.

METHOD AND MATERIALS

Keratin Stripping Technic

For convenience, the flexor aspect of the forearm has been chosen by most investigators. Half inch wide cellophane tape 6 to 8 cms. long is applied to the flexor aspect of the forearm and the edges outlined by a ball point pencil so that subsequent pieces of tape may be accurately applied to the same area. The tape is smoothed on with the finger, then one end is lifted and pulled off quickly. This is repeated until the surface of the skin becomes shiny and glistening when viewed obliquely. The surface becomes slightly reddened but not moist. It was found that if the strips of cellophane tape are pulled off from opposite ends alternately, a smaller number of strips were required (1). The number of strips necessary to denude the keratin from the epidermis varies from about 10 to 30 strips. Blond, fair-haired people sometimes requiring only 8 to 10 strips.

A. On three volunteers, two separate areas were stripped. One area was left uncovered after stripping. On the other area, the last remaining cellophane strip was left in place. Two millimeter biopsies were taken at 24, 48 and 72 hours with the aid of 1% xylocaine local anesthetic. In the cellophane covered area, biopsies were taken through the tape, care being used not to disturb the surrounding cellophane. Including controls, a total of 16 biopsies was taken. The tissues were fixed in Bouin's solution for 24 hours, dehydrated and embedded in paraffin. Serial sections were cut at 8 microns, stained with hematoxylin and eosin, and examined with oil emersion objective (980 × magnification) and a square aperture eyepiece. All cells were counted and measured according to the technic published by Pinkus (10) in 1952.

B. Four biopsies were taken at 48 and 72 hours from the normal appearing epidermis, 3 to 4 mm. away from the stripped skin. These biopsies were fixed and sectioned in the same manner but only the mitoses were counted. Judging from previous work of Pinkus (9, 10) and the first studies in this report, the average field (980 × magnification) 100 microns in diameter using a square aperture eyepiece contains approximately 80 to 100 basal and prickle cells. The number of mitoses per field can be readily determined and the percentage of mitoses per 100 basal and prickle cells can then be calculated. Using this method for obtaining the percentage of cells in mitosis, it is important to keep constant as many factors as possible. The magnification, the size of the field and the thickness of the tissue are of prime importance. In sections which are not cut vertically to the surface or in the presence of acanthosis, the method is not applicable. In spite of all these drawbacks, it was found to be a useful method to quickly ascertain the per-

TABLE 1

A decrease in mitoses occurred in the keratin stripped area covered with cellophane tape

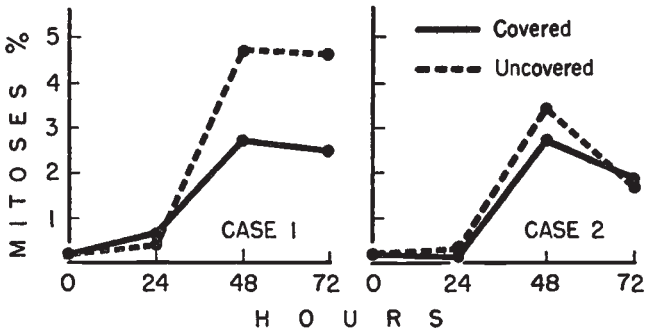
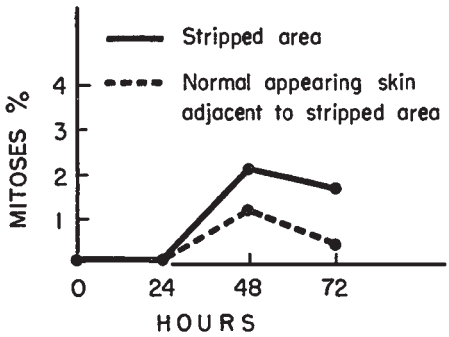


TABLE 2

After keratin stripping, an increased number of mitoses was found in the adjacent epidermis



centage of mitotic figures and has given fairly constant results. The figures shown in Table 2 were obtained in this manner and are lower than those in Table 1 which are more accurate, being corrected by the use of Abercrombie's formula (10).

C. The procedure followed for the histochemical demonstration of acid phosphatase was reported by Rutenburg and Seligman. This procedure is a post-incubation coupling technic using sodium-6-benzoyl-2-naphthyl phosphate. Eight biopsies were studied by this technique, controls 24, 48 and 72 hours, after keratin stripping. Fresh frozen sections were used.

Four biopsies, a control, 24, 48 and 72 hour specimens were obtained after stripping and examined for the presence of succinic dehydrogenase by the technic published by Pearson (7, 8). Fresh tissue was frozen immediately to −70°C. and sectioned at 5 microns in a Linderstrom-Lang Cryostat (7,(8). Nitro-neotetrazolium salt (8) was used in these determinations which is claimed to give more specific results than some of the other tetrazolium salts previously used.

EXPERIMENTAL OBSERVATIONS

A. The peak of mitosis usually occurs at 48 hours but it appears that in cases which are stripped too vigorously there is a delay in mitotic count which does not reach a peak until 72 hours. The number of mitoses in biopsies from the uncovered area and from the cellophane covered area are illustrated in

two of the cases in Table 1. There is a decrease in the number of mitoses in the biopsies from the covered area compared to biopsies taken at the same time from the uncovered skin.

B. In an effort to study how far the mitotic unrest spread from the keratin stripped epidermis, 2 mm. biopsies were taken from apparently normal skin, 3 to 4 mm. from the stripped site. Including the width of the punch, the biopsies represented areas 3 to 6 mm. away from the stripped skin. In all biopsies, normal stratum corneum was present, thus confirming the clinical impression that this area was not denuded of stratum corneum. Biopsies taken at 48 hours and 72 hours showed an increased mitotic count which in most cases was only slightly less than the mitotic count of the stripped skin.

C. Control sections of normal skin and biopsies taken at 24, 48 and 72 hours after stripping were examined by the histochemical method for acid phosphatase by post-incubation coupling technic of Rutenburg and Seligman (13, 14), and confirmed their findings in normal skin. No change was found in the concentration of the formazan dye in the basal or prickle cells during the period of epidermal regeneration.

No staining of the epidermal cells in normal controls or after stripping occurred in the sections treated by the histochemical technic for the demonstration of succinic dehydrogenase (Pearson (8)).

DISCUSSION

Studies on epidermal regeneration after keratin stripping, like any biological study has an inherent margin of error. Technical as well as personal interpretation factors contribute a certain degree of error. Uneven stripping may lead to a considerable difference in the mitotic counts and too much stripping apparently causes excessive cellular damage and a delayed mitotic response. Another source of discrepancy in the results obtained may occur from the fact that the number of mitoses vary from section to section of the same biopsy specimen. The mitoses in a section of an apparently well stripped area may be few in number while other sections of the same biopsy may contain many. The various factors responsible for the burst of mitoses after keratin stripping were fully discussed by Pinkus (9, 10). Direct cellular damage and the simple loss of keratin and its replacement by the so-called "feed-back" mechanism were discussed.

Besides the trauma of the stripping procedure itself, dehydration of the skin due to loss of keratin may contribute to further cellular damage, hyperemia and infiltration in the corium (10). This dehydration effect may be minimized by two methods. 1) applying a greasy ointment base. 2) or by applying an occlusive dressing. Pinkus and Steele (11) demonstrated that there is a slight decrease in the total number of mitotic figures after applying a bland ointment base. This finding would seem to indicate that the prevention of excessive drying minimized the overall stimulus of keratin stripping. The results of keeping an occlusive dressing on the stripped skin showed a decrease in mitoses, which would also seem to indicate a decrease in the stimulating effects of dehydration through

loss of keratin. Wells (17) showed that hydrocortisone ointment tended to decrease the mitotic response compared to the application of a plain ointment base. He suggested that the keratin stripping technic may prove to be a good tool for biological assays by comparing the effects of various drugs in ointment vehicles to the action of hydrocortisone ointment on the perivascular lymphocytic infiltration in the corium. Hydrocortisone treated stripped areas show less perivascular lymphocytic accumulation.

Table 2 illustrates the results of biopsies taken from normal appearing skin adjacent to the stripped area. From these findings it is apparent that the mitotic unrest spreads out from the site of keratin stripping to a distance of at least 3–6 mm. How far this response spreads out beyond 6 mm. into the surrounding epidermis was not determined. The spread of mitoses may be due to minimal indirect trauma to the surrounding epidermis while keratin stripping, or possibly the traumatized actively mitotic cells affect the surrounding epidermal cells by direct contiguity. Another possibility is that the spread of mitoses may be associated with the triple response of Lewis (2). After stripping an area too vigorously or applying an irritating substance to the surface of the stripped skin an increased redness and slight edema occurs in this area and a red flare appears around it.

The pitfalls in the interpretation and demonstration of cellular enzymes by histochemical methods are many. Incubation times used to demonstrate acid phosphatase in fresh frozen sections were ½, 1, and 1½ hours. There was no consistent change in the concentration of the blue staining dye in the epidermis of the control biopsy or at 24, 48 or 72 hours. Therefore it would seem evident that with the histochemical technic used, there is no change in the concentration of the enzyme acid phosphatase during active mitosis of the epidermal cells. The sections treated in this manner showed the same findings in the epidermis and corium previously reported by Moretti and Mescon (5, 6). The maximum staining occurred in the region of the stratum granulosum at short incubation periods, with longer incubation periods, diffuse staining occurred throughout the epidermis.

Succinic dehydrogenase was not demonstrated in the epidermis of biopsies of normal or stripped skin although there was good deposition of fine particles of dye in the upper ⅓ of the external root sheath, the hair bulb as well as the eccrine ductal and glandular cells. Previous workers (4, 12), have obtained different results demonstrating considerable quantities of succinic dehydrogenase in the epidermis using other tetrazolium salts. Nitro-neo-tetrazolium salt produces a fine deposition of dye and appears to give consistent results (8).

SUMMARY

1. By means of the keratin stripping technic, the effect of covering the stripped skin with cellophane tape as an occlusive dressing was found to cause a decreased mitotic response as compared to the uncovered stripped area. Besides cellular trauma, the effect of dehydration due to loss of stratum corneum appears to be one of the factors responsible for the mitotic response.

2. Normal-appearing epidermis 3 to 6 mm. from the stripped skin showed an increase in mitosis. The stratum corneum in each section studied appeared to be normal in thickness, thus confirming the clinical impression that the stratum corneum was intact.
3. When compared to control studies, no change in the concentration of the enzyme acid phosphatase could be demonstrated after keratin stripping.
4. No succinic dehydrogenase was found in the epidermal cells in normal or stripped skin on histochemical examination using nitro-neo-tetrazolium salt.

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